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Thesis

# MORPHOLOGY OF THE ADRENAL GLAND OF MICE

## (PRELIMINARY TO A COMPARATIVE MORPHOLOGY)

BY

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# I-INTRODUCTION

In recent experiments on the validity of endocrine research on the domestic Norway Rat, Richter (1946) proposed that the domestic rat which is commonly used for all types of research in endocrinology and allied fields "may be nothing more than an artificial creation of the laboratory experimenter produced by a long period of selective breeding under unnatural conditions." Richter based this statement on comparisons which were made between domestic Norways and many thousand wild Norway rats recently trapped from the streets and alleys of a large city. His study revealed the existence of great differences in the anatomy and function of the adrenal glands.

Since marked differences in adrenal structure were found between wild and domestic rats, the thought occurred that similar differences might exist in such closely related animals as domestic and wild mice. A survey of the literature to date has revealed that all experimental work with mice has been concerned with the domestic mouse. Since the laboratory mouse is very much used in endocrine research it seems that a comparison of wild and domestic varieties is necessary in order to ascertain whether the differences found by Richter (1946) for rats are present in mice.

It is the purpose of this thesis to collect all available information on the morphology of the adrenal glands of the laboratory mouse in order to form the basis for a future comparative morphology of the adrenal gland of wild and domestic mice.

This preliminary study will involve a survey of the literature concerning the structure and development of the adrenal gland of the laboratory mouse and its structural modifications accompanying various aspects of the reproductive cycle, as well as a brief summary of experimental methods used in a morphological study of the mouse adrenal.

## II- REVIEW OF LITERATURE

### The Development of the Adrenal of the Laboratory Mouse to Twenty-five Days After Birth

The literature on the development of the laboratory mouse adrenal is quite complete. Inaba (1891) was the first to describe the development of the mouse adrenal. Since then Fusari (1893) , Roud (1902) , Soulié (1903),and Waring (1935), have confirmed his account as to its early development. Of them, Waring gave the most complete description of the embryonic development. Many other workers have traced the development of the mouse adrenal from the time of birth to maturity, with special emphasis on the development of the inner zone of the cortex and sex differences ( Masui and Tamura, 1926; Howard-Miller, 1927; Deanesly, 1928; Howard, 1930; Gersh and Grollman, 1939; Bartels, 1941; and McPhail and Read, 1942 ).

#### The Cortical Anlage

The earliest adrenal anlagen were observed in the embryo of twelve days ( Inaba, 1891; Waring, 1935 ). At this stage Waring describes serial transverse sections showing a group of cells immediately above the peritoneum in the region where the nephric tubules pass towards the Wolffian duct. The cortical anlage is situated in the angle of the mesentery between the aorta and the germinal ridge. ( figure 1 ).

Due to the twisting of the embryo of this age Waring was unable to define the position of the anlage exactly in relation to the segmentation of the embryo. Such an exact location would be difficult since the cell mass making up the adrenal anlage is distinguishable from the surrounding mesenchyme only by the closer aggregation of its nuclei and its more densely crowded cytoplasmic granules. Inaba (1891), however, was able to locate the anlage more accurately. He placed it at about the middle of the anterior two segmental tubules to the sixth and seventh tubule. According to his description, the anlage was consolidated and separated from the peritoneum by the thirteenth day.

Fusari (1893) and Soulié (1903) also submitted evidence to support Inaba and Waring in regard to the mode of origin and age of appearance of the adrenal anlage. Roud (1902) described three anlagen in the eleventh day, genital, adrenal and prevascular, as proliferations of the mesothelium. He stated that by the thirteenth day the adrenal anlage had lost its connection with the peritoneum.

## The Medullary Anlage

Waring (1935) observed that on the thirteenth day of foetal life a definite sympathetic mass was closely applied to the cortical anlage. Since this mass was not

seen on the twelfth day and was well established on the thirteenth, he concluded that it was very quickly developed and that from this sympathetic mass sympatho-chromaffin elements would eventually migrate into the cortical anlage to form the medulla of the adult adrenal. Inaba (1891) agreed as to the probable origin of the medulla from the frequency of sections which showed actual contact of the medulla and the ganglion cells. He described the fourteenth day as the time of the origin of medullary cells.

### Union of the Anlägen to Form the Adrenal Gland (Fourteenth day embryo to day of birth)

By the fourteenth day the cortical anlage is clearly separated from the general mesenchyme by a well marked mesodermal sheath ( Soulie, 1903; Waring, 1935). Inaba (1891) says separation is complete at thirteen days. At this stage Waring could distinguish the anlage from neighboring tissue by the densely packed state of its large finely granular cells. He characterized the cytoplasm as strongly eosinophilic and the nuclei as large and sausage-shaped.

Inaba (1891) described a type of infiltration in which fibers enter the cortical anlage from the sympathetic mass and run deeply into it, ending in small sympatho-chromaffin cells as is shown in figure 2. Waring

(1935) observed this type of infiltration on the fifteenth day and added that on the fourteenth day he had observed individual deeply staining sympatho-chromaffin elements, unconnected with any nerve fibers, migrate from the sympathetic mass into the spaces of the cortical anlage. He said that nerve fibers could be seen within the organ and entering its substance at different points, but unaccompanied by sympatho-chromaffin cells. He was uncertain as to whether there are two types of migration or whether he had observed two phases of the same type.

From the fourteenth to the sixteenth day of intrauterine life, there seems to be a gradual concentration of the scattered sympatho-chromaffin into a more compact mass in the center of the gland. Migration of the sympatho-chromaffin cells continues accompanied by a multiplication of the medullary cells in situ ( Inaba, 1891; Waring, 1935 ).

By the sixteenth day the gland has enlarged and projects into the coelom, possessing a well-marked capsule (Waring, 1935). During the period from sixteen days to birth there is a further general increase in size and a gradual concentration of the sympatho-chromaffin elements into a more compact central medulla. Also at this time a distinct change is noted in the character of the cortical cells. Many assume features characteristic of

the future fasciculata. They become cuboidal, more vacuolate and can be distinguished from the darker peripheral cells. It is at this time that Waring (1935) first noted indications of the formation of the interlocking zone. He observed that some of the inner cortical cells retained their dark eosinophilic appearance and seemed to intermingle with and surround the sympatho-chromaffin cells.

McPhail and Read (1942) in a later work could not confirm Waring's observations on the appearance of the interlocking or X-zone in the sixteen day embryo. They first noticed such cells at the day of birth.

## The Mouse Adrenal at Birth

The period of gestation in mice is twenty days (Parkes, 1925). Inaba (1891) reports that at birth the medulla is not a compact mass but has cortical cells intermixed throughout its substance (figure 3); however, the distinction of cell types is clear since the nuclei of the medullary cells are smaller that those of the cortical cells. Waring (1935) also obserwes a close interlocking of the cortex and sympatho-chromaffin tissue, with some of the cortical strands extending across the central mass. Just outside the medulla is the interlocking zone, a layer one or two cells thick, characterized by a highly eosinophilic cytoplasm.

Waring (1935) stated that differentiation of the outer portion of the cortex into zona fasciculata and future zona glomerulosa becomes evident at birth, although Masui and Tamura (1926) say that this differentiation of the cortex is not evident until the period from five to fifteen days. The cortex peripheral to the interlocking zone consists of small cells with a distinct radial arrangement, and for the first time blood vessels are noticed running radially from the periphery towards the center of the gland. These vessels tend to separate the cortical cells into radial rows. Waring (1935) describes the cytoplasm of this region as distinctly eosinophilic but not so much so as that of the interlocking zone. The sympatho-chromaffin elements have not as yet attained any clear cellular arrangement, but consist of an association of nuclei surrounded by coarsely granular cytoplasm with no evident cell walls. Both the nuclei and cytoplasm are basophilic.

Second to Eighth Post-natal Day

The general characteristics of the mouse adrenal from the second to eighth post-natal day are centered in a gradual change of the whole gland towards the early mature condition. Waring (1935) observed that towards the end of this period there is a noticeable increase in the proportion of the medulla to the cortex, and the capsule

surrounding the gland becomes more fibrous. He observed a gradual increase in the interlocking zone to a width of two or three cells and a disappearance of the single cortical strands previously notice completely traversing the medulla. Inaba (1891) and Waring (1935) both record a further change in the medulla during this period. Its cord-like arrangement is as yet very weakly developed and the nuclei are about equal in size to those of the cortex. Later in the period they noticed that the medulla had attained a definite cellular structure with cell boundaries. Towards the eighth day they observed a gradual enlargement of the medullary nuclei until they appeared slightly larger than those of the cortex accompanied by a gradual vacuolization of the medulla.

Inaba (1891) observed that for a month after birth the cortical nuclei are gradually decreasing in size and at the same time the medullary nuclei are gaining very slightly. He shows this in the following table giving the average size of the nuclei in microns:

| | 1 Day | 3 Days | 10 Days | 29 Days | Adult |
|---|---|---|---|---|---|
| Medulla | 5.2- | 5.6- | 5.6 | 6- | 6- |
| Cortex | 6.5- | 6.0- | 5.4- | 5- | 5 |

Inaba believed that this change was due to the formation of the cord-like arrangement in the medulla, causing

larger nuclei, and formation of the zona reticularis in the cortex, causing smaller nuclei.

Masui and Tamura ( 1926) noticed that between the cortex and medulla a number of cells containing lipoid appeared, the cytoplasm of which had a strong affinity for eosin. Since this was a condition similar to the reticularis of the mature female, they marked it as the beginning of the zona reticularis.

Between the second and the eighth day the fasciculata shows an increase in size and number and there is an increasing tendency for the fasciculate columns to be separated by blood vessels, emphasizing the radial arrangement of this layer: however, involving no elongation of the cells themselves. Waring (1935) describes the onset of the differentiation of the future glomerular layer towards the periphery of the fascicular columns. There is a gradual indefiniteness to the arrangement of the cells in this area and at the extreme periphery the nuclei are even more densely crowded. Since the glomerular structure is more clearly defined at the periphery, Waring concludes that this zone differentiates from without inwards.

Bartels (1941) described a differentiation of the cortex into a zona glomerulosa which comprised one-half of the cortex at one week, and which gradually decreased

until at three weeks it consisted of a single row of groups of four to eight cells. During the third week the zona fasciculata consisted of from two-thirds to three-fourths of the cortex, with no definite boundary between it and the glomerulosa.

Ninth to Twenty-fifth Post-natal Day

The period from the ninth to twenty-fifth post-natal day is characterized by a progressive withdrawal of the interlocking tissue from the medulla and its formation into a zone surrounding and interlocking with the medulla; a considerable enlargement of this area towards the end of this period; and a beginning of a sex difference in size. All the literature is in agreement on this point even though there are slight variations in the time of origin and maximum growth of this zone.

Waring (1935) found that the interlocking or X-zone first appeared as scattered cells in the sixteenth day embryo, reached its maximum in the male at twenty-one days post-natal life, and continued to develop in the female. Masui and Tamura (1926) and Howard (1939) found an X-zone in their series from five to fifteen days after birth. McPhail and Read (1942) timed the beginning of this area at birth when a few scattered cells appeared. At four days they noticed that one to two layers of cells were

visible, and that from twenty-five to thirty days this area ceased to develop in the male.

Waring (1935) described an inner cortical zone four cells wide at eighteen days, which increased considerably by the twenty-first day, after which it began to show a definite sex difference. The average male had an interlocking zone eight cells wide while that of the female averaged ten cells. On the twenty-first day he observed sections showing isolated parts of the inner cortical eosinophilic tissue stranded within the medulla. Even in the interlocking zone itself, irregular spaces appeared running along the radii of the gland, diverging and giving the impression of a reticular zone. Waring found this less obvious in the female than in the male.

In the male the inner cortical zone remains the same until the twenty-fifth day, whereas in the female this zone grows rapidly, and at the end of this period consists of many layers of cells which are round in shape and contain a small number of minute lipoid granules, as is characteristic of the mature female (Masui and Tamura, 1936). Howard-Miller (1927) also marks twenty-one days as the beginning of a sex difference in the mouse adrenal.

During this period the radial arrangement of the fasciculata zone became more prominent. Waring (1935) noticed that the individual cells themselves were elon-

gating in a radial direction and at twenty-five days he described a reticular arrangement of the cells at the inner ends of the fascicular columns where they contacted the interlocking zone. Waring pointed to this as the beginning of the reticular zone of the adult cortex which was apart from the interlocking zone and was seen at the same time. McPhail and Read (1942) observed no such zone separating from the fasciculata. With the exception of Waring, most writers use the terms, reticularis, X-zone, interlocking zone and inner cortical zone interchangeably.

At approximately eighteen days after birth, Waring (1935) observed that the irregular arrangement of cells peripheral to the fascicular columns became more clearly evident. At the extreme periphery he noticed a close aggregation of nuclei, which at twenty-one days conformed to the arched arrangement of cells typical of the adult glomerular zone. Waring described these cells as crowded and suggestive of rapid multiplication and a tendency to spread inward.

By fourteen days the medullary cells have increased in size and have become arranged in groups bounded connective tissue ( Waring, 1935). These cells continued to enlarge even after twenty-five days. Waring also ob-

served an increase in the size of the medullary nuclei until eighteen days at which time they were distinctly larger than the cortical nuclei. At ten days Inaba(1891) recorded medullary nuclei larger than those in the cortex.

## Sex Differences in the Mouse Adrenal

Sex differences in the mouse adrenal were first described by Masui and Tamura (1926) and were later studied histologically and experimentally by Howard-Miller (1927) and Deanesly (1928), and have since been the subject of a great number of publications. Deanesly (1938) said that no other species so far studied has shown a similar histological distinction between male and female adrenal glands; however, Ingle (1942) stated that in most, if not all, species of mammals the adrenal cortex of the female is much greater in size than that of the male.

### Twenty-five to Thirty-five Days After Birth

From twenty-five to thirty-five days after birth a definite sex difference in the mouse adrenal was observed by all workers in the field. As previously stated the interlocking zone of the male attained its maximum size on the twenty-first day and remained constant until the thirtieth day, while this zone in the female continued to develop (Waring, 1935; Bartels, 1941; Gersh and Groll-

man, 1939; Masui and Tamura, 1926).

Deanesly (1928) said that at three weeks neither the male nor the female adrenal cortex had developed adult characteristics of size and cellular arrangement. During the next two weeks, however, she described both as having approximately reached their adult size. She reported an enlargement of the X-zone of the female which by the fifth week constituted one-half of the cortex. In the male of this same age a partially degenerated cortex was evident and a band of fibrous reticular tissue had formed around the medulla. Deanesly (1938) and Deanesly and Parkes (1937) said that the "essential" sex difference was first noticed at five weeks. In all probability they meant this as the period of the greatest difference.

Howard-Miller (1937) noticed the first sex difference at four weeks. She said that the X-zone in the male ceased to grow at this time and soon disappeared with a variation of ten days. Both Howard-Miller and Deanesly (1928) correlated the disappearance of the X-zone with the time of appearance of sexual maturity of the male and agreed with Bartels (1941) that by the fourth or fifth week in the female, the X-zone comprised the largest part of the cortex.

Waring (1935) describes the process of degeneration of the male inner cortical zone as beginning on the

thirtieth day as an irregular split running around the gland parallel to the outer circumference and between the interlocking zone and the inner layer of the fasciculata. This process becomes more marked by the thirty-fifth day and begins to spread inward.

The Mouse Adrenal After Thirty-five Days

Regardless of the many differences of opinion as to the beginning of a sex difference in the mouse adrenal, practically all workers agree as to the time of degeneration of the X-zone in the male. Waring (1935) states that the interlocking zone ceases to exist as a layer at thirty-seven days. Howard-Miller (1927), Whitehead (1931), and Deanesly (1928) time its disappearance at thirty-eight days and Masui and Tamura (1926) and Preston (1928) at forty. (Table 1). At this time a connective tissue layer which stains blue with Mallory triple, appears around the male medulla.

Waring (1935) describes a reticular zone outside the degenerating area, the cells of which are shorter, denser and take eosin stain more deeply than the fascicular cells. He observes a considerable variation in the formation of this layer. Deanesly (1928) disagrees with Waring and states that the X-zone is a transitory development of the adult zona reticularis.

By the sixth week degeneration of the inner cortical

zone of the male is complete without exception (Bartels, 1941; Waring, 1935; Deanesly, 1928; Masui and Tamura, 1926). This is the final development stage and the mature condition of the male.

In the unmated female the X-zone reaches the peak of its development by the fifth to sixth week (Bartels, 1941; Gersh and Grollman, 1939; Masui and Tamura, 1926). Howard-Miller (1927) states that the structure which is attained by the male before forty days is not attained by the female until from eighty to two hundred days and that at the height of development of both sexes the X-zone constitutes twelve to fifty-five per cent of the cortex. Neither the above nor Deanesly (1928) are able to set an average peak of development or time of degeneration of the X-zone of the unmated female because of the wide variation in this process. (Table 2 ).

Bartels (1941) described degeneration of the X-zone of the virgin female beginning in one-half of his specimens at fifty days and in all cases by ninety-three days. Whitehead (1931) placed the time of disappearance of the X-zone of the female from eighty to two hundred days and said that after degeneration the male and female adrenals were indistinguishable. The following measurements of the various parts of the mouse adrenal

were made by Whitehead (1933 b):

a) Average breadth of permanent cortex:

Males ( 1 month ) 0.2 mm.
Females ( 4 months ) 0.2mm.

b) Average breadth of transitory cortex:

Males ( 1.5 months ) 0.03 mm.
Females ( 1 month) 0.08 mm.
In males the X-zone disappeared in four of eight glands at one month.

c) Average breadth of medulla:

Males and females ( after 5 months ) 0.5 mm.

Masui and Tamura (1926) have made similar measurements:

| | Glomerulosa and fasciculata (width in mm.) | Reticularis (width in mm.) | Medulla (width in mm.) | Total Area ( in sq.mm.) |
|---|---|---|---|---|
| Male | 0.165 mm. | | 0.307 mm. | 0.673 sq. mm. |
| Female | 0.138 mm. | 0.307 mm. | 0.248 mm. | 1.562 sq. mm. |

The Adrenal of the Mature Male

In the zona glomerulosa of the adrenal of the mature male the nuclei of the epithelial cells are round in shape and are closely gathered together, making it difficult to make out the cell membrane. The cells of this zone are smaller than those of the fasciculata and their cytoplasm has a granular appearance. They have taken on

the arched structure characteristic of this zone (Masui and Tamura, 1926; Waring, 1935).

The fasciculat layer consists of radial columns of cells between which are blood vessels. The cells of this layer have approximately the same width but vary in length, those next the glomerulosa being the smallest and those nearest the center the longest ( Waring,1935). Some of the dark staining cells of the original inner zone persist outside the ring of fibrous tissue that has formed around the medulla. These cells form an irregular zona reticularis, which can be seen in large adrenals, but is inconspicuous in smaller ones (Deanesly, 1928).

In the medulla of the adult male adrenal the cells are arranged in groups of six to twelve, bound together by a connective tissue layer (Waring,1935 ). The nuclei have already been described. Waring also describes large blood sinuses in the spaces among the cell groups and in many glands remains of stranded cortical material are still to be seen. The entire gland is encased in a fibrous capsule. (figure 4).

The Adrenal of the Mature Unmated Female

In the adrenal of the mature unmated female the structure of the zona fasciculata, zona glomerulosa and medulla are similar to that of the male.

The main difference is to found in the zona reticularis or inner zone of the cortex which develops considerably in the female. The membrance of the medulla, which usually appears in the male, can never be found in the mature female. The inner cortex is in direct contact with the medulla and, in older females, isolated groups of cortical cells can be found in the medulla (figure 5). The cells of the reticularis are round in shape and considerably smaller than those of the fasciculata. The proportionate size of the X-zone appears to be independent of the size of the gland. In the inner cortex the cell membrane is distinct and the cytoplasm contains a considerable amount of granules. The nuclei are also round and contain two or more nucleoli (Masui and Tamura, 1926; Deanesly, 1928; Howard-Miller, 1927).

Waring (1935) and Whitehead (1933) point out that since the interlocking zone reaches greater dimensions in the female than it does in the male, the connective tissue band remains after its degeneration is correspondingly more extensive in the female. Whitehead says this band is broader in the female until 388 days.

The effects of pregnancy, lactation, the oestrous cycle and ovariectomy on the female adrenal, as well as the effect of castration on the male adrenal, will be discussed in a later section.

## The X-zone of the Mouse Adrenal

As has already been described, the sex differences of the mouse adrenal are centered in the X-zone ( the inner cortical zone ). The variations in this zone during the later development of the mouse adrenal have been the subject of many publications and a source of many conflicting opinions. Howard-Miller (1927) was the first to use the term X-zone. She used it to designate the transitory zone in the adrenal cortex which showed age and sex relationships. Both she and DeanesIY (1928) used this term to distinguish the conspicuous development in the zona reticularis in the young female from that in the adult. Waring (1935), however, used the term "interlocking zone" to distinguish the inner cortical zone, the site of sex differences, from the zona reticularis which he placed just outside the interlocking zone. Waring described the interlocking zone as a differentiation of the original cortical anlage and the reticularis as a derivation of the inner ends of the fasciculata. Deanesly and Howard-Miller noticed only one differentiation: that of the X-zone from the fasciculata.

### Degeneration of the X-zone

Howard-Miller (1927) was the first to describe the degenerative process involved in the progressive diminu-

tion of the X-zone. According to her description, the degenerative process starts gradually and extends progressively to the whole zone in both male and female. The cell contents appear filled with fine globules and groups of three to five cells begin to fuse. Meanwhile the nuclei become very large and contain clumps of densely stained chromatin, or small nuclei may appear with irregular jagged boundaries or in various stages of fragmentation. She describes this process as continuing until the whole area of the X-zone becomes a mass of large, clear, homogenous vacuoles, supported by the reticular stroma, and many of the nuclei disappear. If the nuclei remain, they are pressed to the side of the cells and are distorted in shape. This is followed by a disappearance of the vacuolated zone, with the zona fasciculata and medulla coming together, separated by a definite connective tissue capsule and at times by a two or three cell layer of irregular arrangement, the zona reticularis.

Deanesly (1928) describes the same type of degeneration as Howard-Miller, but adds that in most glands another type of degeneration occurs, in which the X-zone seems to disappear gradually as the result of an inconspicuous process of cell degeneration, beginning either at the outside of the zone or next to the medulla.

She describes the space occupied by the degenerating cells as at first filled with reticular tissue and capillaries distended with blood, and later obliterated. Large fat vacuoles sometimes occur next to the medulla in the latter phase of the process, but they are not a constant feature of the degenerating zone. During the resorption of the X-zone the reticular fibers become more prominent and after considerable degeneration this ring encircles the medulla and persists forming a cortico-medullary boundary as was formed earlier in the male. Deanesly states that these two processes may occur together or alone.

In the female Howard-Miller (1927) describes a regular connective tissue line between the X-zone and the rest of the cortex, becoming thicker as the X-zone is resorbed. Whitehead (1933) and Waring (1935) states that this zone is broader in the female than the male. Waring also agrees with Deanesly and Howard-Miller on the process of degeneration but points out that fatty degeneration which is found in some females is never found in the male.

Since there are differencess of opinion as to what constitutes the X-zone there are similar differences as to what degenerates. Masui and Tamura (1926) speak of the reticular zone and Howard-Miller (1927) of the X-zone.

Deanesly (1928) says that degeneration involves only the inner layer of the X-zone, the outer layer giving rise to the reticular layer. Howard-Miller recognizes the reticularis after degeneration of the X-zone but considers it as the inner ends of the fascicular columns. According to Waring (1935) the interlocking zone degenerates and the reticularis is derived from the inner fascicular layer.

Regeneration of X-zone Following Degeneration

The literature regarding the possibility of a secondary X-zone following the degeneration of the primary zone is quite controversial. Masui and Tamura (1926) were the first to observe a regeneration of the X-zone. They noticed that after most of the cells of the zona reticularis underwent fatty degeneration, some of the remaining tissue began to regenerate and continued to increase until it reached normal development. Howard-Miller (1927), however, considered the degenerative process irreversible and Waring (1935) made no mention of the possibility of a regenerative process following degeneration.

Deanesly (1928) observed a small number of mice in which an area similar to the X-zone had developed around its fibrous remains. Howard (1939) made a similar observation. Deanesly believed that the new cortical

zone was the homologue of the zona reticularis in other adult animals. She suggested the possibility that the new zone was the result of an inward movement of the fasciculata cells; however, she observed no such migration. She agreed to the possibility of the explanation given by Masui and Tamura above and also suggested that the new zona reticularis may have arisen in situ, in the same manner as the zone forming in the male adrenal after castration ( the effects of castration are discussed in a later section.). Whitehead (1933) could find no support for Deanesly's view that a secondary X-zone succeeded the first transitory zone. He suggested that the appearances which Deanesly took to indicate a new zone were identical with those of the permanent cortex in which lipoid had disappeared. Whitehead found the new area to be lipoid free, whereas the X-zone was abundant with lipoid.

McPhail and Read (1942 a) performed experiments in regeneration of the adrenal gland following enucleation and transplantation. They suggested that when the fasciculata was intact, a new X-zone could regenerate; but, when the fasciculata was removed, as in the enucleated glands, or degenerated, as in the transplanted glands, that re-development was less likely to occur. In their experiments they found that in no single case was an area developed that could be described as a true secondary X-zone.

Masui and Tamura (1926) found an example of regeneration of the X-zone following its degeneration during pregnancy; however, they gave no evidence as to what its cause was. McPhail and Read (1942) made a study of regeneration following pregnancy and found that no regeneration occurred in suckling animals. Yet in those which were not permitted to nurse their young, a distinct regeneration occurred. ( In a later section pregnancy and lactation will be discussed in detail.)

The X-zone of the Mouse Adrenal in Relation to Other Animals

The adrenals of mice undergo a peculiar development and show histological differences with sex that do not appear to occur in any other kind of animal (Deanesly,1928; Deanesly,1938: Bartels,1941). They account for these differences by differentiating the X-zone of mice from the zona reticularis, found in most other mammals, since the former shows many structural changes not found in the latter. Deanesly (1928) says that the zona reticularis of all rodents, other than mice, is easily differentiated;whereas, this zone in mice is interlocked with the medulla. She describes the adrenal of female rats as being larger than that of males, but states that they show no obvious histological differences.

Howard (1938),in studying the possibility of an X-zone

in rats, since it occurred in such a closely related species as mice, observed manifestations in the rat adrenal which were homologous to the X-zone development in mice. However, she described this zone in rats as being less conspicuous, involving less tissue and lasting for a much shorter time. She notice that the maximum expression of the X-zone in rats appeared to be of the same order of magnitude as the minimum expression in mice.

McPhail and Read (1942) described the X-zone of mice as distinct from the reticularis of other animals. In experiments with degeneration of the X-zone following testosterone propionate administration, they found that considerably greater quantities were necessary to cause degeneration of the reticularis of the rat than the X-zone of the mouse. From this they suggested that the two layers were not functionally analogous.

Ingle (1942) observed that in most, if not all species of mammals, the adrenal cortex of the female was larger than that of the male. He described the existence of an X-zone in rats as well as mice, and pointed out the difference in size as a sex difference similar to that of mice. He did not know whether the functional activity of the cortex of the female was normally greater than that of the male.

Hickman (1939) mentions the possibility of an X-zone

in the rat and cat which is generally included as part of the zona reticularis. He agrees with Howard-Miller (1927), Deanesly (1928), Howard (1930), and Waring (1935) that the X-zone in the mouse is histologically comparable to a zone in the human suprarenal cortex which develops before birth and degenerates and disappears during the first year of life. Deansely says that except for the larger degenerating cells in the human infant, the process of degeneration is quite similar to that found in the cortex of the female mouse. She says that, in both, the human and the mouse, degeneration is accompanied by the formation of a fibrous band around the medulla. Hickman (1939) finds the greatest development of the X-zone in the human and mouse adrenal.

The development of a zone in the human adrenal similar to the X-zone of the mouse mentioned above was described by Elliot and Armour (1911) and Keene and Hewer (1927). They described the foetal cortex, the homologue of the X-zone of mice, developing simultaneously with the true cortex. Both began in the twelve millimeter embryo; however, the foetal cortex developed rapidly and became most active from the twelfth to the twenty-second week of foetal life, and ceased to develop at birth. They described the disappearance of this zone in the human infant during the first twelve months of post-natal life.

## The Reproductive Cycle and the Mouse Adrenal

That there have been structural differences in the mouse adrenal accompanying changes in the reproductive cycle has been the theme of much of the literature concerning the mouse adrenal. As to what these changes are and how they are related there is much diversity of opinion.

### The Effect of Pregnancy and Lactation on the Adrenals

Masui and Tamura (1926) and Tamura (1926) were the first to study the effects of pregnancy and lactation on the mouse adrenal. They noticed that at the beginning of pregnancy degeneration of the zona reticularis began and proceeded until all of its cells disappeared, leaving a small amount of degenerated cells behind; whereas, in the unmated female, this zone continued to grow and reached a peak of development much greater than that of the pregnant female. Many subsequent workers have confirmed Masui and Tamura's work and have made further investigations as to the effect of pregnancy on the mouse adrenal ( Howard-Miller, 1927;Deanesly, 1928; Whitehead, 1933; Waring, 1935; Starkey and Schmidt, 1938; McPhail and Read, 1942 ).

Deanesly (1928) described the adrenal of a mouse

seven days pregnant as containing of a small and vacuolated X-zone with a connective tissue band already formed around the medulla. Adrenals examined in mice eleven to sixteen days pregnant showed only a remnant of an X-zone. Howard-Miller (1927) showed similar results in the following table:

Structure of the Adrenals from Pregnant Animals-

(Ages- Fifty to Seventy-four Days)

| Period of Pregnancy | Total number of cases | % of incidence of degenerating X-zone | % of cases of absence of X-zone |
|---|---|---|---|
| 2-11 | 18 | 89 | 11 |
| 12-17 | 12 | 25 | 75 |

Howard-Miller also found that when pregnancy occurred in an individual which had previously lost the reticular zone, no further reduction occurred.

Tamura (1926) noticed that the most rapid degeneration of the zona reticularis occurred at an early period of pregnancy, before the formation of the placenta. This is contrary to the work of McPhail and Read (1942) who reported that degeneration was most marked in the last third of pregnancy and in the post partum period. At the end of pregnancy, Tamura observed hypertrophy of the medulla and glomerulosa and an increase in the fasciculata. He

found mitotic figures in every zone except the zona reticularis at an early period of pregnancy.

Practically all workers state that pregnancy hastens the production of the same structural condition of the adrenal in the female as occurs in that of the adult male. Masui and Tamura (1926) and Tamura (1926), however, describe a slight difference in the two in that the medulla of the female is somewhat smaller than that of the male. Howard-Miller (1927) said that all structural conditions of the X-zone may be found during pregnancy without specific correlation to certain stages of pregnancy. However, she did not explain how an early pregnancy has the effect of causing a premature degeneration of the X-zone.

Following pregnancy and the ensuing premature degeneration of the X-zone, Masui and Tamura (1926) and McPhail and Read (1942) observed a regeneration of this zone. Although Deanesly (1928) noticed a regeneration of the X-zone of unmated females she recorded nb such secondary X-zone in the pregnant female.

McPhail and Read (1942) found that preventing lactation by destroying the young at birth would precipitate a regenerati on of the X-zone. In their experiments no regeneration occurred from twenty-one to twenty-six days post partum in suckling animals; yet, regeneration

was good in the majority of non-sucking animals even as early as twelve days following parturition. In one mouse they observed a regeneration after the second pregnancy. McPhail and Read concluded that during the early life of the animal the X-zone could show cyclic degeneration with, and repair following, pregnancy. However, they said that if this did occur it would be limited by the onset of the normal process of degeneration. They described the new zone as identical with the primary X-zone in its staining reaction and histological details.

The Effect of the Oestrous Cycle on the Adrenal

Masui and Tamura(1926), Howard-Miller (1927) and Deanesly (1928) described considerable variation in size of the female adrenals during various periods of the oestrous cycle. Both Howard-Miller and Deanesly found all types of adrenal conditions associated with all stages of the oestrous cycle. From this they concluded that the histological changes in the adrenal cortex of the unmated female mouse adrenal were in no way correlated with the various stages of the oestrous cycle.

Masui and Tamura (1926) suggested a relationship between the various stages of the oestrous cycle and the adrenal gland. They reported that in mice just prior to sexual maturity the adrenals grew extremely,and

became twice as large as those of the males, while during pregnancy and lactation they were considerably reduced in size. They related the alternation of degeneration and regeneration of the zona reticularis to the cyclic changes in the ovary. However, they made no report as to what specific changes in the adrenals were related to the various periods of the oestrous cycle.

The Effect of Castration on the Male Adrenal

All writers agree that castration of the male during the existence of the X-zone causes its persistence and continued growth until it reaches the proportions found in the unmated female ( Masui and Tamura, 1926; Deanesly, 1928; Martin, 1930; Gersh and Grollman, 1939; Howard, 1939; Ingle, 1942 ).

Howard (1939) described a mouse castrated at three weeks in which the enlarged X-zone had been retained as long as four months, after which it degenerated and disappeared. Howard castrated some mice at five days of age and noticed that a tremendous impetus was given to the development of the X-zone. At sixteen days such mice had an X-zone which constituted one-third or more of the width of the cortex.

If mice are castrated after they reach sexual maturity, the enlargement of the X-zone proceeds much slower

than in those castrated before. The X-zone of such mice never attains the dimensions characteristic of the mature female. By the end of eight weeks an increase of twenty-five per cent is noticed ( Deanesly,1928; Howard, 1939 ).

Howard said that in animals castrated after maturity, differentiation did not resemble the primary X-zone in all respects; therefore, she referred to it as the secondary X-zone. She observed that in no case did the secondary X-zone interdigitate with the medulla as did the primary X-zone, but was separated by connective tissue. Although their organization was different she described similar cell types for both the primary and secondary X-zones. In general she noticed that the differentiation of a secondary X-zone was the greatest and lasted the longest in animals castrated at thirteen to sixteen grams body weight.

Masui and Tamura (1926) observed that following castration the cells of the male X-zone grow almost as much as, and are similar to those of the female. They noticed that the average breadth of the medulla of castrates was fifteen per cent smaller than that of controls. In castrated males a reduction of twenty-three per cent in the glomerular and fascicular zones occurred. They found that the male adrenal of castrates was ninety-one per cent greater than that of the controls. The following table

gives a comparison of the breadth of various zones of both normal and castrate males and mature females:

| | Glomerulosa and fasciculata ( breadth in mm.) | Reticularis (breadth in mm.) | Medulla (breadth in mm.) | Total Area (in sq. mm.) |
|---|---|---|---|---|
| Male | 0.165 mm. | - | 0.302mm. | 0.673 sq.mm. |
| Female | 0.138 mm. | 0.307 mm. | 0.248mm. | 1.562 sq.mm. |
| Castrate | 0.127 mm. | 0.470 mm. | 0.256mm. | 1.287 sq.mm. |

Deanesly (1928) did not notice a reduction in the medulla following castration, but stated that the medulla "takes no appreciable part in the enlargement." She noticed an enlargement in the male adrenal of forty per cent in thickness in some up to a size equal to that of the female. Deanesly found that the developing X-zone in the male did not appear to be formed by an inward movement of the cells of the two outer cortical zones, but that the new zone appeared at the site of the normal zona reticularis and spread slowly outwards. This is opposite to the view of Waring (1935) that the X-zone develops independently of the normal zona reticularis.

Cramer and Horning (1937) found that when adult male mice were castrated the volume of the medulla charged

with adrenaline underwent a marked diminution.

The Effect of Ovariectomy on the Female Adrenal

In general, ovariectomy experiments on female mice have produced no noticeable effect in the structure or size of the adrenal gland. From this, most authors have concluded that growth and differentiation of the female adrenal is not dependent upon the presence of the ovary (Masui and Tamura, 1926:Tamura, 1926; Howard-Miller, 1927; Deanesly, 1928; Gersh and Grollman, 1939; Hickman, 1939).

Masui and Tamura (1926) found that after ovariectomy, a decrease of fifteen per cent occurred as compared with the adrenal of the normal female. However, they included some large female glands in their controls which tended to emphasize a decrease in size of the female adrenal following ovariectomy. Masui and Tamura stated that apart from this variation, only a negligeable decrease was noticed. They made the following measurements:

| | Glomerulosa and fasciculata (breadth in mm.) | Reticularis (breadth in mm.) | Medulla (breadth in mm.) | Total Area (in sq. mm.) |
|---|---|---|---|---|
| Spayed female | 0.131 mm. | 0.283 mm. | 0.236 mm. | 1.329 sq. mm. |
| Normal female | 0.138 mm. | 0.307 mm. | 0.248 mm. | 1.562 sq. mm. |

## The Effect of Certain Endocrine Secretions on the Adrenal

In order to study the function of the X-zone of the mouse adrenal and to determine its relation to the reproductive cycle, many workers have studied the effects of certain endocrine secretions on this gland. Since in the normal male, the X-zone disappears at maturity, it was thought that the male gonads were causative agents of the degeneration. After the administration of testosterone or other androgenic steroids, there occurred a rapid disappearance of the X-zone in normal and castrated males as well as in immature, nulliparous and ovariectomized females ( Martin, 1930; Cramer and Horning, 1937; Deanesly and Parkes, 1937; Starkey and Schmidt, 1938; Howard, 1939; Gersh and Grollman, 1939; McPhail and Read, 1942, 1942 a).

McPhail and Read (1942) observed that after degeneration of the X-zone resulting from administration of testosterone, regeneration occurred. In some mice they noticed that the regenerated zone was smaller than the primary X-zone, but that no distinction could be made as to the type of cell and staining reaction of the two areas.

Martin (1930) found no change in the mouse adrenal after short period injections ( three to eight days) of standarized preparations of oestrin, corpus luteum, testicular hormone and the luteinizing and gonad stimulating hormones of the anterior pituitary.

Cramer and Horning (1937 ) found that no anterior pituitary or oestrogenic hormones produced a degeneration of the X-zone.

The prolonged injection of oestrin into immature castrate males and normal ovariectomized females brought about a total degeneration of the X-zone. In normal immature males, continued injections of oestrin caused persistence of the X-zone or a feminization of the character of the adrenal gland. Upon prolonged administration of oestrin in the normal adult male the X-zone reappeared (Martin, 1930). Martin also made prolonged injections of corpus luteum and luteinizing hormones without noticing a change in adrenal structure. Following extended injections of the gonad stimulating hormone, he observed that the X-zone increased in size in the immature normal and spayed female and that it peristed in the immature normal male.

Because of the inhibiting effect of the testicular hormone on the X-zone of the mouse adrenal, Starkey and Schmidt (1938) and Cramer and Horning (1937) point to this hormone as being responsible for normal X-zone disappearance. Cramer and Horning state that the X-zone is absent in the male adrenal because, under the influence of testosterone, the medullary cells maintain their full load of adrenaline. They account for its presence in young fe-

male mice, even when sexually mature, because in the absence of the male sex hormone the peripheral cells of the medulla do not maintain their full load of adrenaline. They claim that the increased formation of male sex hormones in female mice as age advances, is the cause of the final disappearance of the X-zone.

Idzkowsky and Starkey ( 1942) experimented with ovarian transplants in the ears of males castrated from twenty-four to twenty-eight days. They noticed that instead of the usual prolonged development of the castrate X-zone to a condition characteristic of females, a marked reduction of this zone was evident. In several such animals they observed a complete disappearance of the X-zone accompanied by a capsule of connective tissue about the medulla, a structural condition quite similar to that of the non-castrate male mouse. From this, Idzkowksy and Starkey concluded that the ovaries secrete a male hormone after transplantation to the ears of castrate males and that this hormone is directly responsible for the reduction of the X-zone. They stated that the maintenance of the seminal vesicle and prostatic tissue during the period of the ovarian transplants confirmed their view as to the androgenic

activity of the ovary.

Martin (1930) suggests that in the non-castrate male the X-zone is inhibited by the presence of the testicular hormone and that in the castrate male, normal and spayed female, its presence is dependent upon the gonad stimulating hormone of the anterior pituitary. Leblond and Gardner (1938) say that the inhibitory effect of testosterone on the X-zone may be mediated through the depressing effect of this androgen on the pituitary. Gersh and Grollman (1939) state that degeneration of the X-zone that occurs normally at the time when the testes begin to function, may not be caused by an "atrophy of disuse," but may be secondary to the general metabolic changes which take place in the whole organism at puberty. Howard (1938) claims an androgenic function of the X-zone based on the fact that the time when the X-zone develops rapidly in the mouse coincides with the period when the accessory sex glands show a disproportionate rate of growth. In discounting the androgenic theory of X-zone function, Gersh and Grollman (1939) state that the same rate of growth of at least some of these glands is maintained in the complete absence

of X-zone tissue.

Gersh and Grollman (1939) believe that the X-zone produces the same cortical hormone that is produced by the other portions of the adrenal cortex and that it differs from the other zones in that it reacts less readily to stimulation and is depressed more easily. They consider the X-zone as less active cortical tissue which reacts to an increased demand by the organism for cortical hormone.

## The Vascular Pattern of the Mouse Adrenal

Very few workers have investigated the vascular pattern of the mouse adrenal, with the exception of the zona fasciculata where the radial arrangement of blood vessels is quite obvious. In the literature on the mouse adrenal, the only thorough study of the vascular pattern is that of Gersh and Grollman (1941) who injected the vessels with India ink and made serial sections reconstructing the vascular pattern.

### The Vascular Pattern of the Normal Mouse Adrenal Containing X-zone

In the capsule of the normal mouse adrenal containing X-zone, aged three to four weeks, Gersh and Grollman (1941) describe three types of capillary plexuses. A few of these capillaries pass to the adjac-

ent periadrenal fat pads and the majority give rise to branches of a similar caliber which penetrate the adrenal tissue. A third part of this capillary network terminates in vessels which penetrate into the glomerular area and are formed into arches. From these arches the vessels of the fascicular zone take their origin. These are more numerous than those in the glomerular layer and are radially arranged with short vessels connecting the columns according to the pattern described by Deanesly (1928), Waring (1935) and others working with the mouse adrenal. As these capillaries reach the X-zone, they become slightly wider and finally join the larger-calibered junctional vascular system of the X-zone. In this zone there are large intervals between the channels which gradually converge into the medullary veins ( Gersh and Grollman, 1941).

The medulla is characterized by wide, thin-walled vessels which exhibit a great deal of distensibility. There is a gradual convergence through four orders of medullary veins into the central vein which passes through the cortex receiving small veins and some cortical capillaries. The arterial circulation of the medulla is derived from a narrow central artery which, after passing through the cortex with the massive vein, breaks up into smaller vessels in the spaces between the medullary veins. These vessels terminate as extremely thin capillaries in the

medullary veins and also in the X-zone. The capillary network of the medulla is much looser than that of the cortex. Within the cortex the X-zone is the least vascular area ( Gersh and Grollman, 1941).

## The Vascular Pattern of the Normal Adult Mouse Adrenal Without X-zone

In the adrenal of the normal adult mouse without X-zone there is not much change in the vascular pattern except for a rearrangement of the cortical capillaries. There is an increase in the straight radial arrangement and in numbers of the fascicular capillaries. At the terminal end of these capillaries the transverse connecting channels become especially prominent and upon entering the much reduced reticularis form a close perimedullary network. These vessels converge towards the collecting veins of the medulla and pass through as many as five orders of vessels in much the same manner as those seen in the younger adrenals (Gersh and Grollman, 1941). Deanesly (1928) describes a similar prominence of the vessels in the reticular area of the adult mouse and Waring (1935) reports the appearance of blood sinuses in the spaces amongst the cell groups of the medulla and reticularis.

The vascular pattern of the mouse adrenal forms the

basis for the organization of the cells of the adrenal cortex of mammals into the parallel cords or columns of the fascicular zone ( Gersh and Grollman, 1941). This radial elongation of the fascicular vessels and cells is seen as taking the place of the apparently haphazard vascular and cellular organization in the early stages of development of the mouse adrenal ( Whitehead, 1933; Waring, 1935).

Methods of Use in a Morphological Study of the Mouse Adrenal

As this is a study of the laboratory mouse adrenal preliminary to a comparative morphology of both domestic and wild varieties, a brief summary of experimental methods useful in such a comparison will be presented.

Methods of Fixing and Staining
The Mouse Adrenal

The methods of fixing and staining sections of the mouse adrenal vary with the individual worker as well as depending upon the type of work to be done. For micro-anatomical detail, most workers use Bouin's fixative; however, Howard-Miller (1927) claims that Zenker's formal and Mueller's fixative produce results equal to that of Bouin's.

For distinguishing cortex and medulla as well as the different layers of the cortex, most workers use various

haematoxylin stains ( Harris', Ehrlich's, Delafield's and iron haematoxylin ) either alone or in combinations with eosin. Such stains show a stronger affinity for the cortex than the medulla. Within the cortex, the X-zone or reticularis shows the greatest affinity for stain ( Howard-Miller, 1927; Deanesly, 1928). In my own work, I have found that Mayer's haemalum and eosin, used in combination with orange G, produce the clearest distinction between cortex and medulla.

Howard-Miller (1927) found that by using Sudan III alone, the fasciculate cells would stain deeply and the inner cortical cells would be untouched. By this method she easily distinguished between the fascicular and reticular layers. Whitehead ( 1931, 1953 a) suggested a combination of haemalum and eosin followed by Mallory III, in order to ascertain the presence of a connective tissue band around the medulla. If present it would stain blue. He also used a combination of haemalum and Sudan III in order to determine the lipoid content of the mouse adrenal.

## Methods of Determining Relative Volumes of the Layers of the Adrenal

The normal mouse adrenal is so small that any method of weight determination or volumetric measurements would be too liable to error for use in experimental

work. Deanesly (1931) employed the paper reconstruction method in order to determine relative volumes of the cortex and medulla. Because of the variability in size of female adrenals, she used only males. After making serial sections of the mouse adrenal parallel to the largest plane, she projected every other section on paper of uniform thickness at a magnification of twenty-five diameters and made tracings of the outline of the medulla and cortex for each. She then ascertained the weights in paper of the cortex, medulla and the whole gland; and, from a comparison of volumes of several selected sections with their corresponding paper weights, she worked out the volume of the cortex and medulla for each gland, expressed in cubic millimeters. Blumenfeld (1939) worked out the following formula for determining the volume of each gland:

$$V = \frac{\text{Wt. of paper outline x thickness of gland}}{\text{Wt. of paper per unit area x magnification}^2}$$

In order to determine the weight of paper per unit area he weighed ten sample pieces, each a hundred square centimeters in area, at ten times during the year. Howard (1938) also used the paper weight method, but traced only every seventh section at sixty-five diameters.

Deanesly ( 1931) and Daughady (1941) found that

the volume of the gland could be determined from the area of the largest section. From her previous determinations by the paper reconstruction method, Deanesly found the ratio of paper weight to volume. She found that the volume of any gland could be estimated by multiplying the product of the number of sections and the paper weight in grams of the largest section by a constant. From a comparison of her two methods, Deanesly found them both proportionate, the second and shorter method showing a maximum error of eight per cent for the whole gland and eight to twelve per cent error for the cortex. Carlson (1931) found a similar discrepency when comparing the single and serial section reconstruction methods.

Dornfield, Slater and Scheffè (1941) found that the volume of small organd could be most accurately determined by use of a planimeter. After making serial slides, they traced the projected outline of each section with the planimeter to find the area of the magnified section. They reduced the magnified area to the actual area and multiplied the latter by the thickness of each section in order to ascertain its volume. The total volume equaled the sum of the volumes of all the sections.

Dornfield, Slater and Scheffè have also outlined a method of calculating volume by use of selected sections. By plotting the areas of the camera lucida sketches against

their serial numbers and measuring the area under the curve by use of an planimeter, they found that they actually measured the volume of the entire organ except for a constant factor depending on magnification and thickness of the sections. Using coordinate paper ruled twenty lines to the inch, they let the horizontal units represent the serial number of the sections and the vertical units represent the areas of the camera lucida sketches in square inches. By means of the curve thus constructed, they calculated the volume of the organ from the last planimeter reading P by the following formula:

$$V = \left[\frac{P\ N\ Y\ (645)}{M^2}\right] \qquad \left[\frac{t}{1{,}000}\right]$$

V= volume of the organ in cubic millimeters

P= planimeter reading under the curve in square inches

M= linear magnification of the camera lucida projection

t= thickness of each section in micra

N= number of sections per horizontal inch on the graph

Y= square inches per vertical inch on the graph

Donaldson (1935) used the silhouette method for comparing the volumes of the cortex and medulla of the adrenal gland. Following removal, the adrenals were fixed and clear-

ed and silhouettes of the cortex and medulla were traced on paper by passing a bright light through the cleared gland. By holding each gland in several different positions and making tracings of each position, he could fairly accurately compare the volume of the different glands. Donaldson stated that the value of this method was in being able to compare the volume of small organs without making serial sections. He said that later, if necessary, the glands could be sectioned for a study of histological detail.

## Comparison of Body Weight and Length With the Size of the Adrenals

There have been several attempts to correlate body weight and length of the mouse with the size of the adrenal. Tamura (1926) made such a study and found that no correlation existed. He described a wide variation of adrenal size for each weight group studied.

Tepperman, Engel and Lang (1943) made a similar study of rats and found that in animals above one hundred grams, there was a linear relationship between body weight and adrenal size. In females, he found a larger adrenal size but stated that the correlation still existed. He suggested that a similar correlation might exist in the mouse.

Hett (1926), in a study of fifty male mice, reported

that the medulla size was proportional to the body weight; however, he found a considerable variation in the ratio of the cortex and body weight ( figure 6). The cortex showed marked variations in mice above five grams. In a study of females, Hett described a ratio of medulla and body weight similar to that found in the male, but stated that the variations in the cortex were much greater than in the male.

## DISCUSSION

In the above account of the morphology of the adrenal gland of the laboratory mouse, there are a few inconsistencies, concerning which definite conclusions cannot be drawn until further studies are made. Regarding the nature and origin of the X-zone Waring's (1935) view is opposed to that of most other workers. He describes the X-zone as a differentiation of the cortical anlage, rather than a development of the inner layer of the cortex as is generally thought to be the case. Wheras Howard (1927), Deanesly (1928) and McPhail and Read (1942) descibe the adult reticularis as a derivation of the X-zone, Waring gives the reticularis and X-zone separate origins. However, since most workers are opposed to Waring's view, it would be best to consider the X-zone as originating from the inner layer of the cortex and giving rise to the much reduced zona reticularis of the adult mouse.

Martin (1930), in discussing the effect of oestrin on the X-zone of mice, mentioned that prolonged injections of oestrin into immature castrate males brought about a total degeneration of this zone. This is seemingly inconsistent with his view that the X-zone is inhibited by the presence of testicular hormones in the male, and develops in the female because of the absence of these

hormones. He also reported that administration of oestrin in the normal adult male caused a reappearance of the X-zone. Why would oestrin cause degeneration of the X-zone in immature castrates and regeneration in normal adult males? Seemingly, the lack of the inhibitory effect of the testicular hormones in the castrate male and the addition of oestrin would cause an increase in the X-zone . Martin attempts to explain this by suggesting that in non-castrate mice, the X-zone is inhibited by the presence of the testicular hormone and that in the castrate male, normal and spayed female its presence is dependent upon the gonad stimulating hormone.

The preceding account of the morphology of the adrenal gland of the laboratory mouse has revealed many methods, problems and cautions that will be of value in making a more inclusive comparative morphology.

Because of the greater variations found in the female mouse adrenal than in the male, it would probably be best to limit the comparison, at first, to males only. As previously mentioned, the X-zone of the female adrenal undergoes an extensive growth, beyond that of the male, and is affected by the reproductive cycle to such an extent that it would complicate a beginning comparison of wild and domestic mice. Since the variations in the X-zone of the male adrenal are not as great and last for a much

shorter time than in the female, it would probably be more suitable for beginning a comparison. Also since some writers describe a variation in size between the left and right adrenals, it is essential to keep separate records for each side.

The best age at which to make a comparative study of the male X-zone is at three weeks, the peak of its development. For a study of the male adrenal uncomplicated by X-zone variations it would be best to use mice aged six weeks or older. By this time the X-zone has completely degenerated in the male.

A comparison of the extent of development of the X-zone could be made best in unmated females five to six weeks of age. After this, X-zone variations are even more pronounced due to a difference in the rate of degeneration and the possibility of regeneration of this zone varying with each animal. There is no definite time when a study of the female adrenal without X-zone variations can be made because of the wide divergence in the time of degeneration of the X-zone in the female as shown in table 2.

In making a comparative morphology of the adrenal glands of laboratory and wild mice it will be necessary to raise colonies of both in order to accurately ascertain

the age. In the case of wild mice, however, any adult males trapped, could be used for comparison without knowing the exact age because of the similarity of adrenal structure of all adult males.

One of the most frequent species of wild mice is the white-footed mouse, Peromyscus leucopus noveboracensis, which inhabits the greater part of the Northern United States. Hamilton (1943) gives detailed measurements and descriptions of many wild species but none are as numerous in this part of the country as the above. Also, comparisons could be made with an intermediate type of mouse such as the house mouse, Mus musculus. Many successful attempts have been made at raising colonies of wild mice, although the general opinion is that such mice raised indoors do not exhibit normal fertility and growth is somewhat stunted. Sumner (1916,1924), Seton (1920), Dice (1929), Svihla (1932) and Burt (1940) have raised large colonies of wild mice under laboratory conditions. Dice (1929) describes methods of making cages, proper handling, ways of marking captive wild mice. He also outlines a standard food ration, causing high fertility of both deer and house mice. For handling, he advocates the use of twelve inch finger forceps with which the tail can be grasped.

Burt (1940) describes a longer life span for captive

Peromyscus than for those free in their natural environment. In nature these mice are usually eliminated by predation before they are one year old. In the laboratory many live as long as five years. Svihla records the period of gestation for Peromyscus maniculatus as being from twenty-two to thirty five days. For Peromyscus leucopus the variation is slightly longer, from twenty-two to thirty-seven days.

Svihla also recorded a difference in reaction to ether between Peromyscus maniculatus and Peromyscus leucopus. The first may be exposed to anethesia without fatal results until practically all movements of the limbs have ceased. The latter must be watched very closely and removed from anesthesia before movements have ceased or else consciousness will not be regained. Allen (1922) described a difference in the susceptibility of house mice to anesthesia. He said albinos are generally very susceptible to ether; whereas, brown and black mice are very resistant.

Since the mouse adrenal is too small to weigh or measure directly with accuracy, it will be necessary to use one of the forms of the paper reconstruction method, mentioned in a previous section. The most efficient and accurate method of determining the volume of the mouse

of the mouse adrenal and ascertaining the relative volumes of each layer is that used by Dornfield (1941). He utilized a planimeter, an instrument for measuring the area within a plane figure, for measuring selected sections of the mouse adrenal. A comparison of this method with that of Deanesly (1931) and Blumenfeld (1939) could be made.

## Possible Problems in a Comparative Morphology of Domestic and Wild Mice

The possible probems involved in a comparative morphology of domestic and wild mice would depend upon the degree of difference, if any, that might exist between these two types. As has been previously mentioned, Richter (1946) found great differences in the anatomy of the adrenal glands of wild and domestic rats, the former being three times as large as the latter. The size of the medulla of both were the same, with the main difference in the increased development at the cortex in wild rats.

It should be determined whether the differences found between wild and domestic rats are also present in mice. If such differences are found, a thorough investigation of their nature should be made by repeating with wild mice much of the experimental work already performed on the laboratory mouse.

It would be necessary to determine the existence of

a zone in wild mice similar to the X-zone in the laboratory mouse. If such a zone is found its reaction to castration, ovariectomy and the various phases of the reproductive cycle should be determined and compared with comparable reactions in the domestic mice. A study of the process of development of the adrenal gland of wild mice as well as the possibility of sex differences should be ascertained and compared with that of the laboratory mouse.

Such a study of wild and domestic mice would reveal whether or not the wild mouse, living in a different environment and under different conditions, struggling for its existence, has essentially the same adrenal structure as has the artificially bred laboratory type. If there is a difference in the adrenal structure in mice similar to that found by Richter (1946) for rats, then many endocrinological experiments performed on domestic mice would probably have to be repeated on wild mice.

## SUMMARY

1. The cortical anlage is formed during the twelfth day of embryonic life. The medullary anlage appears from the thirteenth to fourteenth day. By the sixteenth day the gland has become enlarged and projects into the coelom.
2. The first appearance of the X-zone varies from a few days before birth to a week after and it continues to develop until twenty-one days after birth, when it is equal in both sexes. There is disagreement as to whether this zone originates from the cortical anlage, the medulla or the inner ends of the fascicular columns.
3. The X-zone in the male begins to degenerate during the fourth week and disappears by the sixth week. In the immature female this zone reaches its peak during the fifth or sixth week and begins degeneration in from fifty to ninety-three days.
4. Degeneration of the X-zone is brought about by either a process of vacuolization or fatty cell degeneration.
5. The X-zone as seen in the mouse is thought by some to comparable to a similar zone found in rats, cats and man. Others claim it is different in structure and function.

6. During pregnancy the X-zone begins to degenerate prematurely, but if lactation is inhibited a secondary X-zone will regenerate. Some report regeneration of X-zone in the unmated female.
7. The oestrous cycle has no definite effect on adrenal structure.
8. Ovariectomy produces no noticeable change in the X-zone. Castration during adolescence causes the X-zone to increase and probably reach the proportions found in the mature female. Castration at maturity causes a slower development of the X-zone.
9. Testicular hormones cause reduction and possible disappearance of X-zone. Oestrin brings about degeneration of X-zone in gonadectomized animals and gonad stimulating hormones cause increase in X-zone of females and persistence in males.
10. The X-zone is the least vascularized zone of the cortex.
11. The most accurate method of determining the volume of the various layers of the adrenal is to measure the areas of projected sections of serial slides with a planimeter. By this means the total area can be calculated.
12. The adrenal medulla is generally proportional to the body weight, whereas the cortex exhibits marked variations.

## ABSTRACT

### Morphology of the Adrenal Gland of Mice
### (Preliminary to a Comparative Morphology)

Since a previous work has revealed a marked difference in adrenal structure of wild and domestic rats, it was thought that a similar difference might exist in mice.

It is the purpose of this thesis to collect the available information on the morphology of the adrenal gland of the laboratory mouse in order to form the basis for a future comparative morphology of the adrenal gland of wild and domestic mice.

During the pre-natal development of the mouse adrenal the cortical anlage appears on the twelfth day in the angle of the mesentery between the aorta and the germinal ridge. From the thirteenth to the fourteenth day the medullary anlage appears as a sympathetic mass close to the cortical anlage. From this mass sympatho-chromaffin elements migrate into the cortical anlage and gradually become concentrated into a compact mass. By the sixteenth day the gland has become enlarged and projects into the coelom.

The cortical cells gradually take on a slight resemblance to the future fascicular layer and by the day of birth to a week after birth, the inner layer of the cortex begins to differentiate into an interlocking zone,

or X-zone. Some authors think the X-zone differentiates from the cortical anlage and others place its origin in the medulla. By the end of the first week the glomerular layer is seen to differentiate from the outer ends of the fascicular columns.

The X-zone continues to develop until the end of the third week of post-natal life, at which time it is about equal in size in both sexes. In the male this zone persists until about twenty-five to thirty days and then degenerates rapidly, disappearing completely by the end of the sixth week. In the unmated female, however, the X-zone continues to develop until the sixth week, at which time the whole gland may be three times the size of the male gland. The onset of degeneration of the X-zone in the unmated females varies from fifty to ninety-three days. The completion of the process varies from eighty to two hundred days.

Two types of degeneration are seen to occur in the X-zone of the mouse adrenal. In one type the whole area of the X-zone becomes a mass of homogenous vacuoles supported by a reticular stroma. With the collapse of these vacuoles the fasciculata and medulla are brought together, separated only by a connective tissue band. In the other type the X-zone disappears gradually as the result of an

inconspicuous process of cell degeneration along with the formation of a few fat vacuoles.

Some authors attempt to liken the X-zone of the mouse to the zona reticularis of other mammals, especially in the rat, cat and man. They liken it to the foetal cortex of the human embryo which disappears at birth. Others claim this zone in the mouse is distinctly different in structure and function.

The onset of pregnancy hastens the production of the same structural condition in the female adrenal as occurs in that of the male. However, if lactation is inhibited the X-zone will regenerate into a secondary X-zone, limited only by the onset of the normal process of degeneration. Some authors describe regeneration following degeneration in the unmated female.

Since all types of adrenal conditions are to be found associated with all stages of the oestrous cycle, it seems that there is no particular connection between the two. Likewise, ovariectomy produces no noticeable change in adrenal structure.

Castration of males during the existence of the X-zone causes the persistence and continued growth of this zone until it reaches the proportions found in the unmated female. If mice are castrated after maturity the enlargement of the X-zone proceeds much slower and never attains

the dimensions characteristic of the mature female.

Administration of testicular hormones cause a rapid disappearance of the X-zone in normal and castrated males as well as in immature, nulliparous and ovariectomized females. Likewise, prolonged injections of oestrin into immature castrate males and normal ovariectomized females, bring about a total degeneration of the X-zone. In the normal immature male, oestrin causes peristence of the X-zone and in mature males, reappearance of this zone results. Extended injections of gonad stimulating hormones bring about an increase in X-zone size in immature normal and spayed females and a peristence of this zone in the immature male.

The vascular pattern of the adrenals of mice with and without X-zone are quite similar except that in the first case the vessels giving the characteristic columnar appearance to the fascicular zone become slightly wider as they join the larger-calibered junctional vascular system of the X-zone. The X-zone is the least vascularized part of the cortex.

Since the mouse adrenal is so small it is practically impossible to weigh or measure it directly with accuracy. The most accurate method of determining the volume of the vavious layers of the adrenal is to measure the areas of projected sections of serial slides with a planimeter. By

this means the total area can be calculated.

The size of the medulla in the mouse adrenal is usually proportional to the animals body weight: however, due to marked variations in size the cortex exhibits no such relation to body weight.

Having made a survey of the morphology of the adrenals of laboratory mice, it seems that much of this experimental work needs to be repeated on wild mice in order to ascertain if any difference in adrenal structure similar to that found in rats is evident.

Table 1

Incidence of X-zone Degeneration of the Male

( From McPhail and Read, 1942. Anat. Rec. 84, 51-68).

| Animal Number | Age in Days | X-zone |
|---|---|---|
| 1 | 21 | Well developed X-zone |
| 2 | 26 | X-zone 1/4 cortex |
| 3 | 26 | X-zone 1/4-1/3 cortex |
| 4 | 27 | X-zone 1/4-1/3 cortex |
| 5 | 27 | X-zone 1/4 cortex |
| 6 | 27 | X-zone 1/4 cortex |
| 7 | 27 | X-zone 1/4 cortex |
| 8 | 27 | X-zone 1/4 cortex |
| 9 | 28 | No X-zone |
| 10 | 26 | X-zone 1/3-1/2 cortex |
| 11 | 26 | X-zone 1/2 cortex |
| 12 | 30 | X-zone 1/7 cortex |
| 13 | 31 | Small X-zone 2-3 cells |
| 14 | 31 | X-zone 1/5-1/4 cortex |
| 15 | 32 | No X-zone |
| 16 | 32 | X-zone 1/4 cortex |
| 17 | 33 | X-zone 1/3 cortex |
| 18 | 34 | X-zone 4-6 cells deep |
| 19 | 36 | X-zone 1/3 cortex |
| 20 | 36 | No X-zone |
| 21 | 36 | No X-zone |
| 22 | 36 | No X-zone |
| 23 | 36 | No X-zone |
| 24 | 36 | No X-zone |
| 25 | 36 | No X-zone |
| 26 | 38 | Trace only |
| 27 | 40 | No X-zone |
| 28 | 41 | No X-zone |
| 29 | 41 | No X-zone |
| 30 | 41 | No X-zone |
| 31 | 42 | X-zone, trace only |

Table 1

Incidence of Z-zone Degeneration of the Vela

( From Hoffhall and Read, 1642. Amer. Soc. Ob., 83-86).

| Animal Number | Age in Days | Z-zone |
|---|---|---|
| 1 | 21 | Well developed Z-zone |
| 2 | 24 | Z-zone 1/4 cortex |
| 3 | 28 | Z-zone 1/4-1/3 cortex |
| 4 | 27 | Z-zone 1/3 cortex |
| 5 | 27 | Z-zone 1/4 cortex |
| 6 | 27 | Z-zone 1/4 cortex |
| 7 | 29 | Z-zone 1/4 cortex |
| 8 | 27 | Z-zone 1/4 cortex |
| 9 | 25 | No Z-zone |
| 10 | 22 | Z-zone 1/3-1/2 cortex |
| 11 | 28 | Z-zone 1/2 cortex |
| 12 | 27 | Z-zone 1/2 cortex |
| 13 | 21 | Small Z-zone 2-3 cells |
| 14 | 21 | Z-zone 1/3-1/4 cortex |
| 15 | 25 | No Z-zone |
| 16 | 23 | Z-zone 1/4 cortex |
| 17 | 22 | Z-zone 1/3 cortex |
| 18 | 24 | Z-zone 2-3 cells deep |
| 19 | 22 | Z-zone 1/3 cortex |
| 20 | 22 | No Z-zone |
| 21 | 26 | No Z-zone |
| 22 | 24 | No Z-zone |
| 23 | 22 | No Z-zone |
| 24 | 23 | No Z-zone |
| 25 | 24 | No Z-zone |
| 26 | 26 | Traces only |
| 27 | 23 | No Z-zone |
| 28 | 21 | No Z-zone |
| 29 | 22 | No Z-zone |
| 30 | 21 | No Z-zone, traces only |

Table 2

Incidence of X-zone Degeneration in the Virgin Female

( From Howard-Miller, 1927. Am. J. Anat. 40, 251-293).

| Midpoint of age group | Average Body weight | X+T | S+D+d | O | Total number of individuals |
|---|---|---|---|---|---|
| 20 | 6.7 | 100 | - | - | 7 |
| 33 | 13.3 | 86 | 14 | - | 7 |
| 50 | 15.3 | 41 | 59 | - | 17 |
| 68 | 17.4 | 39 | 61 | - | 18 |
| 93 | 20.7 | 8 | 89 | 3 | 36 |
| 131 | 24.7 | - | 71 | 29 | 17 |
| 170 | 27.7 | - | 62 | 38 | 13 |
| 201 | 26.8 | - | 40 | 60 | 5 |

X= X-zone- no traces of degeneration
T= X-zone- 6 or under degenerating cells
S= X-zone- 6-$\frac{1}{2}$ of cells degenerating
D= X-zone- $\frac{1}{2}$ or more cells degenerating
d= small degenerate X-zone
O= only traces of degenerate X-zone or absence.

Figure 1.- Diagrammatic transverse section, showing position of the cortical anlage in 12 day old embryo: a, adrenal anlage; c, cardinal vein, d, dorsal aorta; g, germinal ridge; m, mesonephric tubule; W, Wolffian duct (Redrawn from Waring, 1935, p. 355).

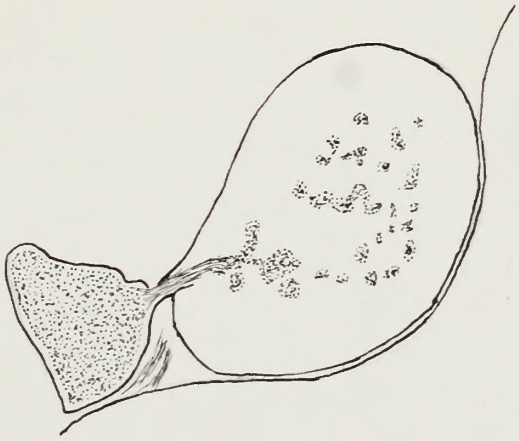

Figure 2. - C.S. of adrenal of a 16 day embryo, showing sympathetic ganglia at the left and the sympatho-chromaffin situated in cortex (Redrawn from Inaba, 1891, p. 288).

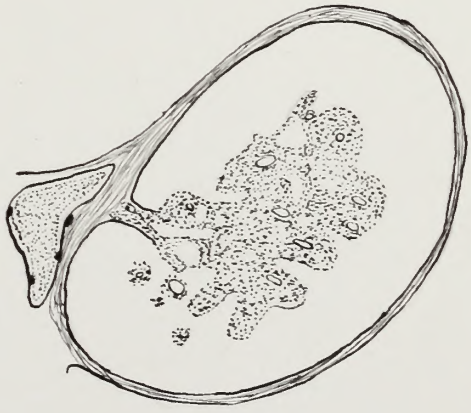

Figure 3. - C.S. of adrenal of a 1 day old mouse, showing the organization of the sympatho-chromaffin elements into a central medulla (Redrawn from Inaba, 1891, p. 219).

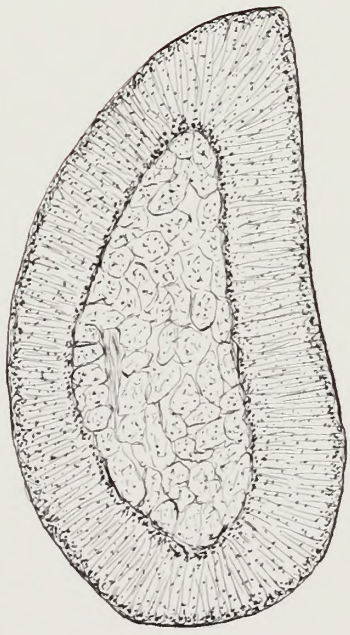

Figure 4. - Section through a large adrenal from a 12 weeks' male, showing fibrous band around the central medulla, and a small, darkly stained reticularis adjacent to it. (Drawn from a photograph by Deanesly, 1928).

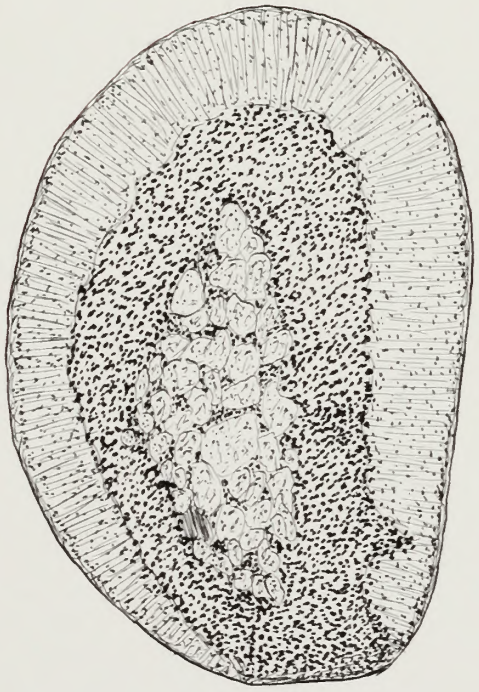

Figure 5. - Section through adrenal of a 12 weeks' old unmated female, showing the large darkly stained X-zone, and groups of cells belonging to it lying free in the medulla (Drawn from a photograph by Deanesly, 1928).

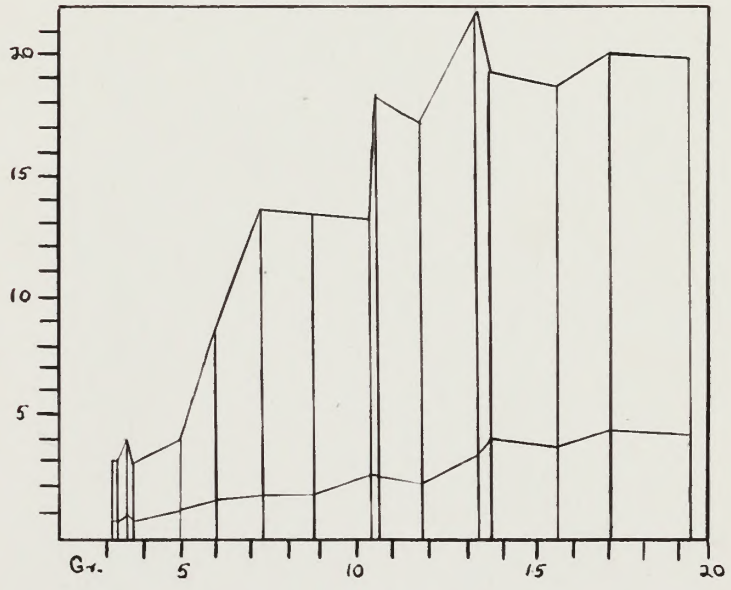


Figure 6.- Graph showing relation of cortex and medulla to body weight of the mouse. The lower curve represents the medulla and the upper curve, the cortex (Redrawn from Hett, 1926).

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